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Gramatzki, Dorothee ; Kickingereder, Philipp ; Hentschel, Bettina ; Felsberg, Jörg ; Herrlinger, Ulrich ; Schackert, Gabriele ; Tonn, Jörg-Christian ; Westphal, Manfred ; Sabel, Michael ; Schlegel, Uwe ; Wick, Wolfgang ; Pietsch, Torsten ; Reifenberger, Guido ; Loeffler, Markus ; Bendszus, Martin ; Weller, Michael

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Dorothee Gramatzki, MD
Philipp Kickingereder, MD
Bettina Hentschel, PhD
Jörg Felsberg, MD
Ulrich Herrlinger, MD
Gabriele Schackert, MD
Jörg-Christian Tonn, MD
Manfred Westphal, MD
Michael Sabel, MD
Uwe Schlegel, MD
Wolfgang Wick, MD
Torsten Pietsch, MD
Guido Reifenberger, MD
Markus Loeffler, MD
Martin Bendszus, MD
Michael Weller, MD

Correspondence to Dr. Gramatzki: dorothee.gramatzki@usz.ch

ABSTRACT

Objective: To explore an association with survival of modifying the current standard of care for patients with newly diagnosed glioblastoma of surgery followed by radiotherapy plus concurrent and 6 cycles of maintenance temozolomide chemotherapy (TMZ/RT → TMZ) by extending TMZ beyond 6 cycles.

Methods: The German Glioma Network cohort was screened for patients with newly diagnosed glioblastoma who received TMZ/RT → TMZ and completed ≥6 cycles of maintenance chemotherapy without progression. Associations of clinical patient characteristics, molecular markers, and residual tumor determined by magnetic resonance imaging after 6 cycles of TMZ with progression-free survival (PFS) and overall survival (OS) were analyzed with the log-rank test. Multivariate analyses using the Cox proportional hazards model were performed to assess associations of prolonged TMZ use with outcome.

Results: Sixty-one of 142 identified patients received at least 7 maintenance TMZ cycles (median 11, range 7–20). Patients with extended maintenance TMZ treatment had better PFS (20.5 months, 95% confidence interval [CI] 17.7–23.3, vs 17.2 months, 95% CI 10.2–24.2, $p = 0.035$) but not OS (32.6 months, 95% CI 28.9–36.4, vs 33.2 months, 95% CI 25.3–41.0, $p = 0.126$). However, there was no significant association of prolonged TMZ chemotherapy with PFS (hazard ratio [HR] = 0.8, 95% CI 0.4–1.6, $p = 0.559$) or OS (HR = 1.6, 95% CI 0.8–3.3, $p = 0.218$) adjusted for age, extent of resection, Karnofsky performance score, presence of residual tumor, O^6-methylguanine DNA methyltransferase *(MGMT)* promoter methylation status, or isocitrate dehydrogenase (*IDH*) mutation status.

Conclusion: These data may not support the practice of prolonging maintenance TMZ chemotherapy beyond 6 cycles.

Classification of evidence: This study provides Class III evidence that in patients with newly diagnosed glioblastoma, prolonged TMZ chemotherapy does not significantly increase PFS or OS.
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GLOSSARY

CI = confidence interval; **GGN** = German Glioma Network; **HR** = hazard ratio; ***IDH*** = isocitrate dehydrogenase; **KPS** = Karnofsky performance score; ***MGMT*** = O^6-methylguanine DNA methyltransferase; **OS** = overall survival; **PFS** = progression-free survival; **RT** = radiotherapy; **STROBE** = Strengthening the Reporting of Observational Studies in Epidemiology; **TMZ** = temozolomide.

Glioblastoma is an intrinsic brain tumor with an annual incidence of 3 per 100,000 individuals worldwide. Patients eligible for multimodality treatment commonly have biopsy or resection as feasible and then postoperative radiotherapy (RT) plus concomitant and 6 cycles of maintenance temozolomide chemotherapy (TMZ/RT → TMZ).[1–3] At a population level, median overall

Supplemental data at Neurology.org

From the Department of Neurology and Brain Tumor Center (D.G., M.W.), University Hospital Zurich and University of Zurich, Switzerland; Department of Neuroradiology (P.K., M.B.), University Hospital of Heidelberg; Institute for Medical Informatics, Statistics and Epidemiology (B.H., M.L.), University of Leipzig; Departments of Neuropathology (J.F., G.R.) and Neurosurgery (M.S.), Heinrich-Heine University Düsseldorf; Division of Neurooncology (U.H.), Department of Neurology, University Medical Center Bonn; Department of Neurosurgery (G.S.), University of Dresden; Department of Neurosurgery (J.-C.T.), Ludwig Maximilians University Munich; Department of Neurosurgery (M.W.), University Medical Center Hamburg-Eppendorf, Hamburg; Department of Neurology (U.S.), Knappschaftskrankenhaus Bochum-Langendreer, Ruhr-University Bochum; Clinical Cooperation Unit Neurooncology (W.W.), German Cancer Consortium, German Cancer Research Center, and Neurology Clinic and National Center for Tumor Diseases, University Hospital Heidelberg; Department of Neuropathology (T.P.), DGNN Brain Tumor Reference Center, University of Bonn Medical School; and German Cancer Consortium (G.R.), German Cancer Research Center Heidelberg, Partner Site Essen/Düsseldorf, Düsseldorf, Germany.

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survival (OS) has markedly improved from the pre-TMZ to the TMZ era.[4] A few smaller, single-institution, retrospective studies claimed prolonged survival of patients with glioblastoma who received extended TMZ treatment beyond 6 cycles.[5–8] The major limitation of all these studies, beyond the retrospective nature, is the comparison of patients who were treated with at least 7 cycles of TMZ to patients who received ≤6 cycles and therefore to patients who in most cases stopped TMZ because of tumor progression. Long-term administration of TMZ was associated with an acceptable safety profile in patients diagnosed with World Health Organization grade III and IV gliomas who received long-term TMZ treatment for at least 12 cycles.[9] Preliminary data from a large pooled analysis of 4 clinical trials (EORTC26981-NCIC CE.3, Radiation Therapy Oncology Group 0525, EORTC26071-CENTRIC, CORE)[3,10–12] indicate that extended treatment with TMZ beyond 6 cycles is not associated with improved OS but with improved progression-free survival (PFS) (hazard ratio [HR] 0.8, 95% confidence interval [CI] 0.6–1) compared to patients who received exactly 6 cycles of TMZ, especially in patients with O^6-methylguanine DNA methyltransferase *(MGMT)* promoter–methylated tumors (HR = 0.6, 95% CI 0.5–0.9).[13] Here, we performed a similar analysis in patients enrolled in the German Glioma Network (GGN), a prospective cohort study, with a focus on the role of *MGMT* promoter methylation status and extent of residual disease after 6 cycles of adjuvant TMZ.

METHODS

Standard protocol approvals, registrations, and patient consents. The GGN is a prospective cohort study involving 8 clinical centers at university hospitals in Germany that included 2,002 patients diagnosed with glioblastoma from October 2004 to October 2010 (http://www.gliomnetzwerk.de). Informed consent was signed by all patients. This study was approved by the review committees of the participating centers.

The primary research question of the present study was to explore a survival benefit from extending maintenance TMZ chemotherapy beyond 6 cycles in patients with newly diagnosed glioblastoma who received TMZ/RT → TMZ. The study is rated Class III because of the absence of randomization and because of differences in baseline characteristics of treatment groups.

This manuscript was prepared in accordance with Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.[14]

Patients and tumors. The present study evaluated clinical features and survival data in patients with newly diagnosed glioblastoma who received TMZ/RT → TMZ (n = 142) with at least 6 cycles of maintenance chemotherapy without progression. The number of cycles was left to the discretion of the sites, and no recommendation was made as part of the GGN cohort study. Diagnosis of glioblastoma was confirmed according to the World Health Organization classification of tumors of the CNS[15] by central pathology review, and clinical data were collected as described.[16] The methylation status of the *MGMT* gene promoter region was assessed by methylation-specific PCR.[17] Analyses for isocitrate dehydrogenase *(IDH)* 1 or *IDH2* gene mutations were performed by DNA pyrosequencing,[18] or by immunohistochemistry for IDH1-R132H protein. Formaldehyde-fixed paraffin-embedded sections 4 μm thick were cut from the tumors. After deparaffinization and antigen retrieval, mutated IDH1-R132H protein was detected by the murine monoclonal mutation–specific antibody clone H09 (Dianova, Hamburg, Germany) with the ultraView Universal DAB detection kit and a Benchmark XT immunostaining system (Ventana-Roche, Mannheim, Germany). Extent of resection was determined by early (<72 hours) postoperative imaging by MRI or by CT when MRI was not feasible or not available. Treatment decisions were made by the treating physicians, patients, and their families, commonly without awareness of the *MGMT* promoter methylation status; 35 patients were included in previous publications.[16,19–25]

MRI scans or at least written reports were collected at the time point when 6 cycles of TMZ chemotherapy were completed to assess tumor status. MRI scans of 36 patients were available for central radiology assessment (P.K., M.B.) according to Response Assessment in Neuro-Oncology criteria. Written neuroradiology reports were provided from 51 additional patients. Data on residual tumor were available from a total of 87 patients. Residual tumor was defined by contrast-enhancing lesions.

To avoid bias that occurs when groups to be compared are determined during study follow-up, all patients who had died or had progressive disease before completion of 6 cycles TMZ were excluded.

Statistics. PFS and OS curves were generated with the Kaplan-Meier method and compared by the log-rank test. PFS was calculated from the day of surgery until progression, death, or end of follow-up. OS was measured from the day of surgery to the date of death or end of follow-up. At the time of last follow-up, all patients who had not died were censored. The χ^2 and Fisher exact tests were performed for analysis of nominal variables, and the Student t test was performed for quantitative variables. Cox proportional hazards regression models were used for univariate and multivariate analyses to test the association of clinical factors, residual tumor burden after 6 cycles of maintenance TMZ, and *IDH* mutation status and *MGMT* promoter methylation status with outcome. For the multivariate model, patients who had complete information on all tested covariables were included (age, extent of resection, Karnofsky performance score [KPS] at the time of diagnosis, *MGMT* promoter methylation status, *IDH* mutation status, residual tumor after 6 cycles of maintenance TMZ). A landmark analysis with a 7-month landmark was performed to estimate landmark PFS and landmark OS after the end of the 6 cycles of maintenance TMZ. All statistical tests were 2 tailed. A value of $p = 0.05$ was defined as statistically significant. All statistical analyses were performed with IBM (Armonk, NY) SPSS Statistics version 24.0.

RESULTS

Patient characteristics. The principal patient characteristics are summarized in table 1 and table e-1 at Neurology.org. A total of 142 patients with newly diagnosed glioblastoma were identified in the

Table 1 Summary of patient characteristics

	Group A, 6 cycles of TMZ	Group B, ≥7 cycles of TMZ	p Value
No.	81	61	
TMZ cycles, n			
Median	6	11	<0.001
Range	6–6	7–20	
Age at diagnosis, y			
Median	58	55	0.359
Range	23–77	27–74	
Age classes, n (%)			
≤65 y	69 (85.2)	51 (83.6)	0.797
>65 y	12 (14.8)	10 (16.4)	
Sex, n (%)			
Male	47 (58.0)	37 (60.7)	0.752
Female	34 (42.0)	24 (39.3)	
Extent of resection, n (%)			
Gross total	42 (59.2)	29 (48.3)	0.420
Incomplete	21 (29.6)	24 (40.0)	
Biopsy	8 (11.3)	7 (11.7)	
No data	10	1	
KPS at enrollment, n (%)			
≤80	37 (53.6)	18 (38.3)	0.105
90–100	32 (46.4)	29 (61.7)	
No data	12	14	
MGMT promoter methylation status, n (%)			
Methylated	43 (59.7)	34 (56.7)	0.723
Unmethylated	29 (40.3)	26 (43.3)	
No data	9	1	
IDH1/2 status, n (%)			
Mutant	7 (9.7)	10 (17.9)	0.179
Wild-type	65 (90.3)	46 (82.1)	
No data	9	5	
Residual tumor,[a] n (%)			
Yes	16 (35.6)	22 (52.4)	0.114
No	29 (64.4)	20 (47.6)	
No data	36	19	
Residual tumor,[a,b] n (%)			
Yes	7 (46.7)	12 (57.1)	0.535
No	8 (53.3)	9 (42.9)	
No data	66	40	

Abbreviations: *IDH* = isocitrate dehydrogenase; *MGMT* = O^6-methylguanine DNA methyltransferase; KPS = Karnofsky performance score; TMZ = temozolomide.
[a] After 6 cycles of maintenance TMZ chemotherapy.
[b] Only patients available for central radiology review.

GGN database (figure e-1). Patients were divided into 2 groups defined by the number of TMZ cycles: 6 cycles (group A, n = 81) and ≥7 cycles (group B, n = 61). The median number of TMZ cycles in group B was 11 (range 7–20). Table 1 shows that both groups were balanced for age ($p = 0.359$), sex ($p = 0.752$), extent of resection ($p = 0.420$), and KPS ($p = 0.105$). The *MGMT* promoter methylation status of the tumor was available in 132 patients: 77 patients had *MGMT* promoter–methylated tumors (58.3%); 59.7% (group A) vs 56.7% (group B) of patients demonstrated a methylated *MGMT* promoter ($p = 0.723$). The high proportion of *MGMT* promoter–methylated tumors reflects the selection bias induced by studying patients who received at least 6 cycles of TMZ. The *IDH* mutation status was available in 128 patients: 7 patients in group A (9.7%) had *IDH*-mutant tumors vs 10 patients in group B (17.9%) ($p = 0.179$). Data on residual tumor (contrast-enhancing tumor; written neuroradiologic reports or central neuroradiologic assessment) at completion of 6 cycles were available in 45 patients in group A (55.6%) and in 42 patients in group B (68.9%). Residual tumor after 6 cycles of maintenance TMZ was described in 16 patients in group A (35.6%) and in 22 patients in group B (52.4%) ($p = 0.114$). In the subgroup of patients with MRI scans available, central radiologic assessment demonstrated residual tumor in 7 of 15 patients in group A (46.7%) and in 12 of 21 patients in group B (57.1%) ($p = 0.535$).

Outcome data. The median time of follow-up was 77.0 months in the whole patient cohort: 68.4 months in group A and 77.0 months in group B. Median PFS was 20.0 months (95% CI 17.0–22.8) and median OS was 33.2 months (95% CI 29.2–37.1) in the whole patient cohort. Median PFS was 17.2 months (95% CI 10.2–24.2) in group A compared to 20.5 months (95% CI 17.7–23.3) in group B ($p = 0.035$). Median OS was 33.2 months (95% CI 25.3–41.0) in group A compared to 32.6 months (95% CI 28.9–36.4) in group B ($p = 0.126$) (table 2 and figure 1A). *MGMT* promoter methylation was associated with increased PFS ($p < 0.001$) and OS ($p = 0.004$) (figure e-2A). In the subgroup of patients with *MGMT* promoter–methylated tumors, neither PFS (25.9 [group A] vs 22.5 months [group B], $p = 0.377$) nor OS (41.3 vs 36.1 months, $p = 0.649$) differed between groups (table 2 and figure 1B). In patients with *MGMT* promoter–unmethylated tumors, increased PFS was observed in patients with extended TMZ treatment (10.9 [group A] vs 14.9 months [group B], $p = 0.012$), whereas no difference was seen for OS (24.7 vs 26.9 months, $p = 0.132$) (table 2 and figure 1B). Absence vs presence of residual tumor after 6 cycles of maintenance TMZ was strongly prognostic (PFS $p < 0.001$; OS $p < 0.001$) (figure e-2B). However, extended TMZ treatment beyond 6 cycles was not

Table 2 Kaplan-Meier survival data: Subgroup analysis

	No. (events)	Median PFS (95% CI), mo	*P* (log-rank)	No. (events)	Median OS (95% CI), mo	*P* (log-rank)
All patients						
6 cycles of TMZ (A)	81 (77)	17.18 (10.20–24.16)	0.035	81 (69)	33.15 (25.26–41.03)	0.126
≥7 cycles of TMZ (B)	61 (52)	20.49 (17.66–23.33)		61 (44)	32.62 (28.86–36.39)	
Methylated *MGMT* promoter						
6 cycles of TMZ	43 (39)	25.87 (19.72–32.02)	0.377	43 (34)	41.28 (31.23–51.33)	0.649
≥7 cycles of TMZ	34 (27)	22.53 (17.84–27.21)		34 (24)	36.10 (24.58–47.62)	
Unmethylated *MGMT* promoter						
6 cycles of TMZ	29 (29)	10.89 (10.48–11.29)	0.012	29 (27)	24.69 (18.64–30.74)	0.132
≥7 cycles of TMZ	26 (25)	14.92 (9.88–19.97)		26 (20)	26.85 (15.93–37.78)	
No residual tumor[a]						
6 cycles of TMZ	29 (27)	24.98 (18.41–31.56)	0.148	29 (22)	44.69 (34.69–54.69)	0.515
≥7 cycles of TMZ	20 (14)	22.95 (14.55–31.36)		20 (11)	41.38 (10.40–72.35)	
No residual tumor[a,b]						
6 cycles of TMZ	8 (8)	22.00 (9.64–34.36)	0.512	8 (7)	47.21 (27.67–66.75)	0.928
≥7 cycles of TMZ	9 (8)	22.53 (21.47–23.58)		9 (6)	41.38 (10.91–71.84)	
Residual tumor[a]						
6 cycles of TMZ	16 (15)	12.30 (7.28–17.31)	0.461	16 (16)	21.31 (15.34–27.29)	0.597
≥7 cycles of TMZ	22 (21)	14.56 (11.85–17.27)		22 (20)	21.80 (13.48–30.13)	
Residual tumor[a,b]						
6 cycles of TMZ	7 (6)	13.44 (6.71–20.17)	0.956	7 (7)	16.20 (14.26–18.13)	0.904
≥7 cycles of TMZ	12 (12)	13.41 (11.24–15.58)		12 (12)	21.80 (6.44–37.16)	

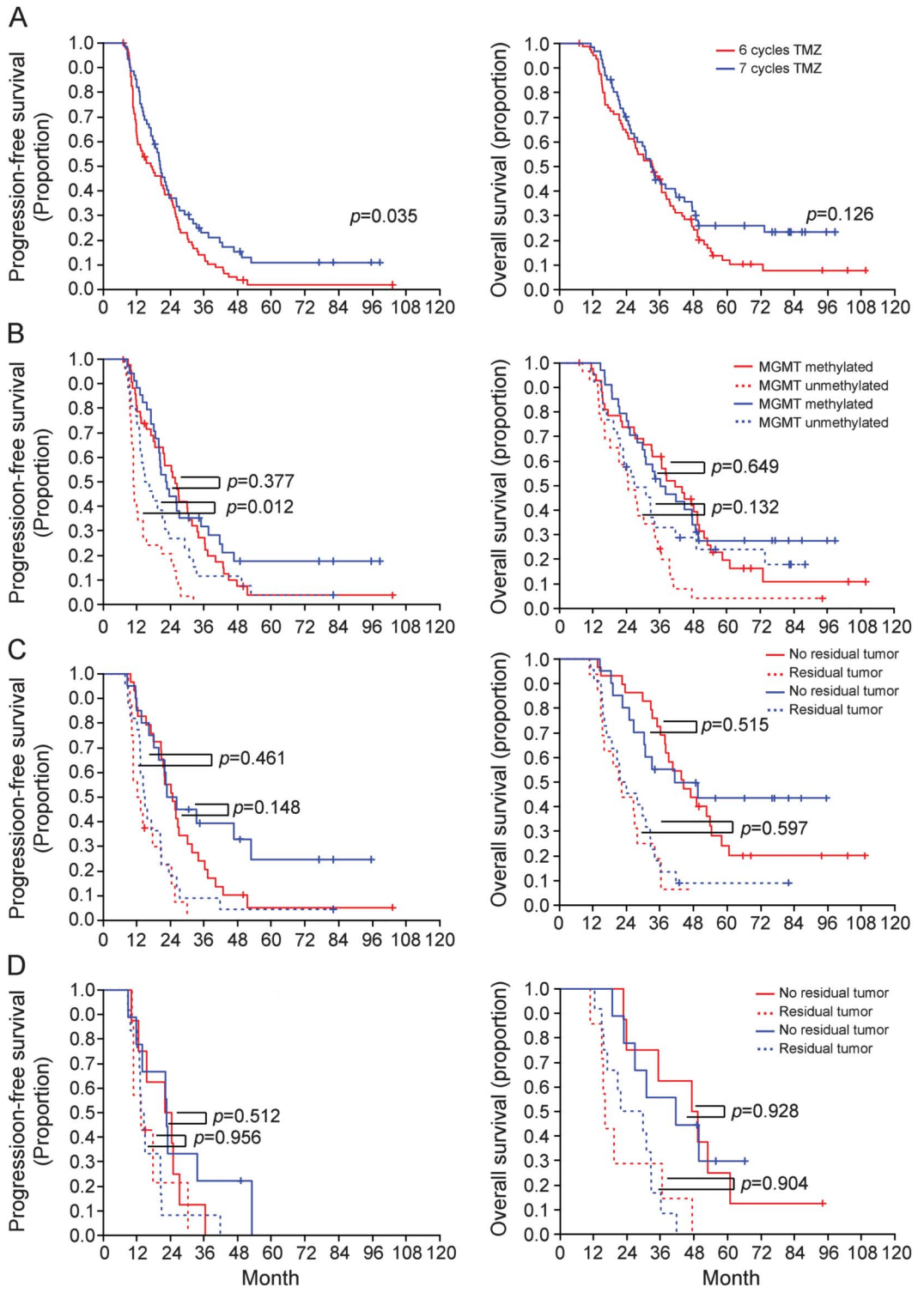
Abbreviations: CI = confidence interval; *MGMT* = O^6-methylguanine DNA methyltransferase; OS = overall survival; PFS = progression-free survival; TMZ = temozolomide.
[a] After 6 cycles of maintenance TMZ chemotherapy.
[b] Only patients available for central radiology review.

associated with outcome in patients with confirmation of residual tumor after 6 cycles of TMZ treatment (PFS 12.3 months [group A] vs 14.6 months [group B], $p = 0.461$; OS 21.3 vs 21.8 months, $p = 0.597$) (table 2 and figure 1C). Similar results were observed when only patients with central reference radiology were included (PFS 13.4 [group A] vs 13.4 months [group B], $p = 0.956$; OS 16.2 vs 21.8 months, $p = 0.904$) (table 2 and figure 1D). Similar outcome by group was also observed in patients without residual tumor (PFS 25.0 [group A] vs 23.0 months [group B], $p = 0.148$; OS 44.7 vs 41.4 months, $p = 0.515$; patients with central reference radiology: PFS 22.0 vs 22.5 months, $p = 0.512$; OS 47.2 vs 41.4 months, $p = 0.928$) (table 2 and figure 1, C and D). The apparent plateau in group B in patients without residual tumor results from a larger number of long-term surviving patients with *IDH*-mutant glioblastomas. For patients without tumor burden after 6 cycles of TMZ and with OS >48 months, *IDH* mutations were observed in 5 of 9 patients in group B and in 2 of 11 patients in group A.

In addition, a 7-month landmark analysis was performed to estimate PFS and OS after the end of the 6 cycles of maintenance TMZ (figure e-3). Median landmark PFS was 10.2 months (95% CI 3.2–17.2) for group A and 13.5 months (95% CI 10.7–16.3) for group B ($p = 0.035$), and landmark OS was 26.2 months (95% CI 18.3–34.0) for group A and 25.6 months (95% CI 21.9–29.4) for group B ($p = 0.126$).

Association of age, sex, extent of resection, KPS, *MGMT* promoter methylation status, *IDH* mutation status, residual tumor, and TMZ cycles with outcome. Patients were divided into 2 groups defined by age, sex, extent of resection, KPS, *MGMT* promoter methylation status, *IDH* mutation status, residual tumor, and TMZ cycles. Univariate analysis using the Cox proportional hazard model was performed to assess their association with PFS or OS. Extent of resection, *MGMT* promoter methylation status, *IDH* mutation status, and residual tumor burden after 6 cycles of maintenance TMZ, but not age, sex, KPS, or extended TMZ treatment, were risk factors for progression. In addition, age, extent of resection, *MGMT* promoter methylation status, *IDH* mutation status, and residual tumor after 6

Figure 1 Association of TMZ exposure with outcome



Progression-free survival (left) and overall survival (right) in patients with newly diagnosed glioblastoma by number of temozolomide (TMZ) maintenance cycles: 6 cycles (group A, red curve) vs ≥7 cycles (group B, blue curve). (A) All patients, (B) patients stratified by O^6-methylguanine DNA methyltransferase (*MGMT*) promoter methylation status, and (C and D) patients stratified by absence vs presence of residual tumor after 6 cycles of maintenance TMZ. (C) All patients with information on residual tumor; (D) only patients available for central radiology review.

cycles of maintenance TMZ, but not, sex, KPS, or extended TMZ treatment, were identified as significant risk factors for survival (table 3).

Multivariate analysis was performed to address whether extended TMZ treatment might be an independent factor associated with survival when adjusted

Table 3 Univariate analysis with regard to tumor progression or death

		PFS		OS	
	No.	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value
Age, y					
≤65	120	1		1	
>65	22	1.40 (0.88–2.23)	0.158	1.89 (1.17–3.04)	0.009
Sex					
Male	84	1		1	
Female	58	0.84 (0.59–1.20)	0.347	0.97 (0.66–1.41)	0.874
Extent of resection					
Gross total	71	1		1	
Incomplete	45	1.03 (0.70–1.52)	0.888	1.01 (0.66–1.55)	0.955
Biopsy	15	2.34 (1.27–4.30)	0.006	3.42 (1.84–6.34)	<0.001
KPS, %					
90–100	61	1		1	
≤80	55	1.28 (0.87–1.88)	0.207	1.45 (0.96–2.19)	0.082
***MGMT* promoter methylation status**					
Unmethylated	55	1		1	
Methylated	77	0.46 (0.32–0.67)	<0.001	0.57 (0.39–0.84)	0.004
***IDH1/2* status**					
Wild-type	111	1		1	
Mutant	17	0.38 (0.21–0.70)	0.002	0.19 (0.08–0.46)	<0.001
Residual tumor[a]					
No	49	1		1	
Yes	38	2.54 (1.60–4.04)	<0.001	3.67 (2.22–6.07)	<0.001
Residual tumor[a,b]					
No	17	1		1	
Yes	19	2.15 (1.05–4.39)	0.036	4.29 (1.88–9.80)	0.001
TMZ treatment					
6 cycles	81	1		1	
≥7 cycles	61	0.68 (0.48–0.98)	0.036	0.74 (0.51–1.09)	0.127

Abbreviations: CI = confidence interval; HR = hazard ratio; *IDH* = isocitrate dehydrogenase; KPS = Karnofsky performance score; *MGMT* = O^6-methylguanine DNA methyltransferase; OS = overall survival; PFS = progression-free survival; TMZ = temozolomide.
[a] After 6 cycles of maintenance TMZ.
[b] Only patients available for central radiology review.

for known prognostic factors in glioblastoma (age, extent of resection, KPS, *MGMT* promoter methylation status, *IDH* mutation status, and residual tumor burden after 6 cycles of maintenance TMZ). Multivariate analysis revealed methylated *MGMT* promoter status (HR = 0.3, 95% CI 0.2–0.6), mutant *IDH* status (HR = 0.2, 95% CI 0.1–0.6), and residual tumor burden after 6 cycles of TMZ (HR = 2.6, 95% CI 1.3–5.4) to be strongly associated with PFS, but not older age (HR = 0.9, 95% CI 0.5–1.9), reduced extent of resection at first surgery (HR = 0.8, 95% CI 0.4–1.6), reduced KPS (HR = 1.2, 95% CI 0.6–2.2), or extended TMZ treatment beyond 6 cycles (HR = 0.8, 95% CI 0.4–1.6) (table 4). In addition, multivariate analysis demonstrated reduced KPS (HR = 2.6, 95% CI 1.3–5.0), methylated *MGMT* promoter status (HR = 0.5, 95% CI 0.3–0.9), mutant *IDH* status (HR = 0.1, 95% CI 0.0–0.5), and residual tumor burden (HR = 3.0, 95% CI 1.5–6.3) to be strongly associated with OS, but not older age (HR = 1.2, 95% CI 0.6–2.5), reduced extent of resection (HR = 0.9, 95% CI 0.4–1.8), or extended TMZ treatment beyond 6 cycles (HR = 1.6, 95% CI 0.8–3.3) (table 4).

DISCUSSION The current standard of care of TMZ/RT → TMZ with up to 6 cycles of TMZ maintenance defined in 2005[3] was based on a phase II study

Table 4 Multivariate analysis, mutually adjusted for all factors examined in this table, with regard to tumor progression or death

		PFS		OS	
	No.	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value
Age, y					
≤65	51	1		1	
>65	12	0.92 (0.45–1.89)	0.820	1.18 (0.56–2.45)	0.671
Extent of resection					
Gross total	36	1		1	
Incomplete/biopsy	27	0.81 (0.41–1.60)	0.536	0.85 (0.41–1.75)	0.656
KPS, %					
90–100	36	1		1	
≤80	27	1.18 (0.63–2.20)	0.615	2.59 (1.33–5.04)	0.005
***MGMT* promoter methylation status**					
Unmethylated	25	1		1	
Methylated	38	0.34 (0.19–0.61)	<0.001	0.47 (0.25–0.91)	0.025
***IDH1/2* status**					
Wild-type	53	1		1	
Mutant	10	0.20 (0.07–0.56)	0.002	0.11 (0.03–0.52)	0.005
Residual tumor[a]					
No	40	1		1	
Yes	23	2.60 (1.25–5.44)	0.011	3.04 (1.46–6.29)	0.003
TMZ treatment					
6 cycles	31	1		1	
≥7 cycles	32	0.82 (0.41–1.62)	0.559	1.58 (0.76–3.26)	0.218

Abbreviations: CI = confidence interval; HR = hazard ratio; *IDH* = isocitrate dehydrogenase; KPS = Karnofsky performance score; *MGMT* = O^6-methylguanine DNA methyltransferase; OS = overall survival; PFS = progression-free survival; TMZ = temozolomide.
[a] After 6 cycles of maintenance TMZ (all patients).

that defined the 6 cycles somewhat arbitrarily.[26] Accordingly, numerous efforts of improving outcome by intensifying adjuvant TMZ have been undertaken, using dose intensification in the first 6 months, prolongation of TMZ maintenance, or both,[12,27,28] all without convincing results to suggest superiority compared with the standard of 6 cycles at 5 of 28 days. Yet, prolonging TMZ maintenance beyond 6 to 12 months or even more has become a common practice, notably in the United States.

Here, we used the GGN cohort to identify 142 patients who completed 6 maintenance TMZ cycles (TMZ/RT → TMZ) without progression and then either were followed up by observation (group A) or continued TMZ maintenance (group B). Patient characteristics in both groups were similar (table 1), but 10 of 17 patients with *IDH*-mutant tumors belonged to group B, and 6 of these 10 patients were long-term survivors (OS > 48 months). Prolonged maintenance TMZ treatment results in better PFS but not OS (figure 1A), whereas survival did not differ between groups A and B analyzed in the group of patients with a methylated *MGMT* promoter (figure 1B). In patients with an unmethylated *MGMT* promoter methylation status, extended TMZ treatment was associated with increased PFS (figure 1B), but there were more *IDH*-mutant patients (n = 5) in this subgroup than in the subgroup of unmethylated patients who received only 6 cycles of TMZ (n = 0) (data not shown). This is different from the preliminary analysis reported by Blumenthal et al.,[13] who found TMZ beyond 6 cycles to be linked to improved PFS in patients with *MGMT* promoter–methylated tumors.

Induction of chemoresistance by continued exposure to TMZ itself may limit a potential benefit from extending maintenance TMZ treatment. There might be a link of a hypermutated phenotype of glioblastoma at the time of recurrence related to TMZ pre-exposure.[29] This hypermutated phenotype involves mutations in mismatch repair pathway genes, which are a pathway of acquired resistance to TMZ in vitro and in vivo.[30,31] Moreover, a hypermutated phenotype of gliomas may result in progression to

a more malignant tumor phenotype at the time of recurrence.[32]

In Germany and other European countries, extended TMZ maintenance is probably most often considered for patients with residual tumor after 6 cycles of TMZ maintenance. This was reflected by the higher number of such patients in group B (35.6% vs 52.4%, $p = 0.114$) (table 1). However, in these patients, no differences in PFS or OS were observed (table 2 and figure 1, C and D). Multivariate analysis confirmed KPS at the time of diagnosis, *MGMT* promoter methylation status, *IDH* mutation status, and residual tumor burden after 6 cycles of TMZ to be associated with survival (table 4), but not age or extent of resection, both associated with survival in the univariate analysis (table 3).

Limitations of this study include its uncontrolled nature, retrospective analysis albeit of prospectively assembled data, and small sample size at least for subgroups. There may have been bias toward prolonging treatment in symptomatic patients thought to be at risk of early progression or in patients in whom there was uncertainty as to whether there was progression or not. However, we conclude that any potential beneficial effect of prolonging maintenance TMZ would be so small that the patient numbers required to demonstrate this superiority in a randomized fashion would be excessive.

This study indicates that in this patient cohort of the GGN, neither PFS nor OS of patients with newly diagnosed glioblastoma was superior when patients received >6 cycles of maintenance TMZ. These data may not support the practice of extending maintenance TMZ chemotherapy, regardless of *MGMT* promoter methylation status or residual tumor after 6 cycles.

AUTHOR CONTRIBUTIONS

D. Gramatzki: design or conceptualization of the study, analysis or interpretation of the data, drafting or revising the manuscript, statistical analysis, accepts responsibility for conduct of research, and final approval. P. Kickingereder: central radiology review, analysis or interpretation of the data, drafting or revising the manuscript, accepts responsibility for conduct of research, and final approval. B. Hentschel: analysis or interpretation of the data, drafting or revising the manuscript, statistical analysis, accepts responsibility for conduct of research, and final approval. J. Felsberg: molecular analyses, analysis or interpretation of the data, drafting or revising the manuscript, accepts responsibility for conduct of research, and final approval. U. Herrlinger, G. Schackert, J.-C. Tonn, M. Westphal, M. Sabel, U. Schlegel, and W. Wick: analysis or interpretation of the data, drafting or revising the manuscript, accept responsibility for conduct of research, and final approval. T. Pietsch: central reference pathology, analysis or interpretation of the data, drafting or revising the manuscript, accepts responsibility for conduct of research, and final approval. G. Reifenberger: molecular analyses, analysis or interpretation of the data, drafting or revising the manuscript, accepts responsibility for conduct of research, and final approval. M. Loeffler: analysis or interpretation of the data, drafting or revising the manuscript, statistical analysis, accepts responsibility for conduct of research, and final approval. M. Bendszus: central radiology review, drafting or revising the manuscript, accepts responsibility for conduct of research, and final approval. M. Weller: design or conceptualization of the study, analysis or interpretation of the data, drafting or revising the manuscript, accepts responsibility for conduct of research, and final approval.

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